REMARKS

Claims 73, 75, 80, 81, 100 to 112, and 115 to 121 are under consideration. By this paper, claim 110 has been cancelled herein without prejudice. Applicants maintain the right to prosecute any cancelled claims in any related application claiming the benefit of priority of the subject application. New claims 125 to 132 have been added. Accordingly, upon entry of this paper, claims 73, 80, 81, 106 to 109, 111, 112, 115, 116 and 121 to 132 are under consideration.

Regarding the Amendment to the Specification

The specification has been amended to reflect the correct deposit date for the deposited biological material. Applicants thank the Examiner for drawing the error to the Applicants' attention. Accordingly, as the amendment was made to correct an informality, no new matter has been added and entry thereof is respectfully requested.

Regarding the Claim Amendments

The amendments to the claims are supported throughout the originally filed specification or were made to address an informality. In particular, the amendment to claim 73 to delete reference to the light chain variable region CDR sequences identical to CDR1, CDR2 and CDR3 of SEQ ID NO:1 is supported, for example, by originally filed claims 1, 2, 7, and 14, and at page 2, line 9, to page 3, line 14. The amendment to claim 106 to recite "80%" identical to SEQ ID NO:1 and "90%" identical to SEQ ID NO:3 is supported as set forth in the record (e.g., page 19, lines 9-15). The amendment to claim 111 to delete reference to CDRs of heavy chain variable region was made so claim 111 is not redundant with amended claim 73. Thus, as the claim amendments are supported throughout the originally filed specification or were made to address an informality, no new matter has been added and entry thereof is respectfully requested.

Regarding the New Claims

New claims 125 to 132 are supported throughout the originally filed specification. In particular, claims 125, which recites that "said light chain variable region sequence has CDR sequences identical to CDR1, CDR2 and CDR3 of SEQ ID NO:1 is supported, for example, by originally filed claim 20, and at page 5, lines 8-21. Claims 126 to 132 are supported, for example, by originally filed claims 1 to 3, and 8, and at page 19, lines 9-15 of the

specification. Thus, as claims 125 to 132 are supported by the originally filed specification, no new matter has been added and entry thereof is respectfully requested.

Regarding the Objections to the Specification

The specification remains objected to due to the alleged absence of x-axis and y-axis labels for Figure 10A and 10B. Also, allegedly, the amendment to correct the units of grams to milligrams at page 27, lines 17-21 of the specification is new matter.

Applicants respectfully point out that the previously filed amendment correcting grams to milligrams at page 27, lines 17-21 corrects an obvious error. In addition to the fact that one of skill in the art knows that the average weight of a mouse is about 20 grams, and therefore that it is impossible for a tumor to weigh 5-8 times more than the weight of the mouse itself, Applicants direct the Examiner's attention to the specification at page 27, lines 23-25, where it is stated that average volume of the corresponding tumors are 126.3 mm^3 and 158.2 mm^3. If each of these tumor volumes correspond to 96.2 and 150.5 grams, the tumor densities would be 0.763 g/mm^3 and 0.951 g/mm^3, respectively. Densities are typically represented as $grams/cm^3$, so multiplying the tumor densities by a factor of 1000, corresponds to densities of 763 g/cm^3 and 951 g/cm^3, respectively. However, it is not possible for tumors to have this density since the most dense element known is osmium, with a density of 22.6 g/cm^3. Accordingly, in view of the fact that a tumor cannot have a density 35-50-fold greater than the most dense element known, it is obvious that the tumor mass indicated in grams at page 27, lines 17-21 is an error that should be milligrams. Consequently, Applicants' previously filed amendment correcting this obvious error does not add new matter.

Furthermore, mice are 65% water (Exhibit C), which means that 65% of the average 20 gram mouse will have a volume of 13 ml (water has a density of 1 g/cm^3). 13 ml corresponds to 13,000 mm^3). Thus, the disclosed tumor volumes of 126.3 mm^3 and 158.2 mm^3 reflect no more than about 0.75-1.5% of the total volume of an average 20 gram mouse. Additionally, originally filed Figure 10A y-axis has increments of 0.05, which reflect increments in grams, not kilograms. Accordingly, Applicants' previously filed amendment corrects an obvious error and therefore does not add new matter.

As to Figure 10 itself, and that control and SAM-6 treated animal data points are both represented as open circles, in view of the fact that the study results and conclusions depicted in Figure 10A are fully described in the specification at page 27, one of skill in the art could clearly "discern the effects of SAM-6 from the control." At page 27, the

specification states that "According to Figure 10a the average weight of tumors of SAM-6 treated mice is 96.2 milligrams, while average weight of tumors of mice treated with the control antibody is 150.5 milligrams. Figure 10b shows that analysis of the volume of tumors corresponds to with the analysis of tumor weight. The average volume of tumors of SAM-6 treated mice is 126.3 mm^3, while average volume of tumors of mice treated with control antibody is 158.2 mm^3." Thus, even if Figure 10 by itself does not distinguish SAM-6 from control, clearly in view of the originally filed specification one of skill in the art would know that the effect of SAM-6 was to reduce tumor mass and size, compared to control.

Applicants clarify that previously submitted Exhibit A was not intended to replace originally filed Figure 10. Applicants reiterate that submission of a revised Figure 10 or additional description of the results may prompt a rejection for addition of new matter. Furthermore, in view of the fact that one of skill in the art would clearly know that the effect of SAM-6 compared to control is fully described in the specification of the originally filed specification, namely that SAM-6 reduces both average tumor mass and size, there is simply no need to amend Figure 10 or the description of the results. Accordingly, Applicants respectfully request withdrawal of the objection.

The specification stands newly objected to due to the addition of allegedly new matter, namely the correction of 2 incorrect amino acid residues in SEQ ID NO:3 from Lys-Thr to Arg-Pro. Allegedly, there is no written description to support the amendment to correct the sequence error

Applicants respectfully point out that this issue was squarely and unambiguously addressed by the court in *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956 (Fed. Cir. 2002). In particular, the court held "that reference in the specification to a deposit may also satisfy the written description requirement with respect to a claimed material," and that "reference in the specification to deposits of nucleotide sequences describe those sequences sufficiently to the public for purposes of meeting the written description requirement." Id. at 965 and 970. In view of the fact that deposited sequences satisfy the written description requirement for those sequences, the referenced deposited biological material (DSM ACC2903) in the specification adequately describes the recited heavy (SEQ ID NO:3) and light chain (SEQ ID NO:1) variable region sequences. Accordingly, the previously filed amendment correcting the sequence error of SEQ ID NO:3 does not add new matter, and the objection must be withdrawn.

The specification stands newly objected to due to an error in the deposit date for the deposited biological material. The specification has been amended to reflect the correct deposit date for the deposited biological material, DSM ACC2903. Accordingly, the ground for objection is moot.

Regarding the Objection to the Claims

Claims 109 and 110 stand objected to due to being duplicates. Claim 110 has been cancelled herein without prejudice. Accordingly, the ground for objection is moot.

REJECTIONS UNDER 35 U.S.C. §112, FIRST PARAGRAPH

ENABLEMENT

The rejection of claims 106 and 123 under 35 U.S.C. §112, first paragraph as allegedly lacking enablement is respectfully traversed. According to the Patent Office, allegedly absent identity of the antigen, it would require undue experimentation to screen antibody variants for binding to antigen.

Claims 106 and 123, and new claims 125 to 132 are adequately enabled. Contrary to the assertion in the Action, additional knowledge concerning the identity of the antigen to which the claimed antibodies beyond what is disclosed in the as-filed application is not required in order to identify antibodies, including variants, that have specific binding for the target antigen.

As previously pointed out in the record, and supported by the previously filed Declaration under 37 C.F.R. 1.132 executed by Dr. Vollmers, in view of the guidance in the specification and the high level of knowledge and skill in the art at the time of the invention relevant to antibodies, variants having the requisite activity could be produced and identified using routine methods disclosed in the specification or that were known in the art at the time of the invention without undue experimentation. Importantly, such methods do not require greater knowledge of antigen identity than that which is disclosed in the as-filed application. To reiterate, in view of the fact that the antigen to which the antibodies and functional fragments bind is expressed by at least one of five well-defined neoplastic cell lines and SAM-6 antibody also binds to the antigen, one of skill in the art could identify variant antibodies and functional fragments without undue experimentation based upon binding, for example, to one or more of the recited cell lines, and optionally confirm binding of the

variant antibody or functional fragment to the antigen to which SAM-6 binds by competitive binding studies with SAM-6 antibody.

To corroborate Applicants position and the previously filed Declaration under 37 C.F.R. 1.132 executed by Dr. Vollmers, as but one example of identifying variant antibodies without use of any greater knowledge of antigen identity than is disclosed in the as-filed application and without undue experimentation, submitted herewith are pages (Exhibit D) from PCT WO 2010/088739 (a complete copy is submitted in the accompanying IDS). Applicants note that PCT WO 2010/088739 discloses, among other things, variant antibodies, antigen identities, and studies of variant antibody binding to target cells that express antigen to which SAM-6 antibody binds, and to antigen. Applicants reference the following specific studies from the published application as merely examples of studies that corroborate that additional knowledge of antigen identity greater than disclosed in the subject application is not essential to identify variants that bind.

Significantly, the studies in Example 14, pages 99-100 are an analysis of 4 different variant sequences of SAM-6 antibody (pages 87-91 describe the sequences of the variants) for binding to several cancer cell lines, including the BxPC-3 cells disclosed in the application. Using conventional FACS or confocal microscopy methods, variant antibody binding was determined and distinguished from the absence of binding (none of the antibody variants bind to control 23132/93 cell lines) using the same BxPC-3 cell line disclosed in the application. The foregoing studies illustrate one exemplary method by which one of skill in the art could identify variant antibodies without undue experimentation and without any more knowledge of antigen identity than that which is disclosed in the as-filed application.

In addition to Example 14, the data in Example 15 (pages 100-101) refer to cell binding studies of various SAM-6 variants, which were converted to the IgG isotype. Again, variant antibody binding to cells that express target, namely A549 and HeLa cells was determined using cell lines, and without the need for additional knowledge concerning the identity of the target antigen greater than what is disclosed in the as-filed application.

In addition to Examples 14 and 15, the data in Examples 11 and 12 (pages 95-97) refer to binding studies of various SAM-6 diabodies to antigen secreted from A549 cells. Once again, antibody binding to antigen was determined using a conventional method (ELISA) and without the need for additional knowledge concerning the identity of the target antigen greater than what is disclosed in the as-filed application.

In sum, the foregoing data convincingly demonstrate that additional knowledge greater than disclosed in the as-filed specification as to the identity of the target antigen to which the claimed antibodies bind is not essential for one of skill in the art to identify variants within the scope of the claims. Consequently, contrary to the assertion in the Office Action the studies corroborate the previously filed Declaration under 37 C.F.R. 1.132 executed by Dr. Vollmers that one of skill in the art could readily identify variants of SAM-6 that bind to antigen without undue experimentation. Accordingly, the claims are adequately enabled under 35 U.S.C. §112, first paragraph, and the rejection must be withdrawn.

The new grounds for rejection of claims 73, 80, 81, 106 to 112, 115, 116 and 121 to 124 under 35 U.S.C. §112, first paragraph as allegedly lacking enablement is respectfully traversed. According to the Patent Office, allegedly the claims lack enablement for binding of variable region heavy chain sequence (SEQ ID NO:3) of SAM-6 antibody alone.

Applicants respectfully direct the Examiner's attention to WO 2010/088739, which discloses the variable region heavy chain sequence alone binding data in question. In particular, the Example 21, pages 103-105, and Figure 28 disclose substantially the same HeLa cell binding data of variable region heavy chain sequence alone, as illustrated in previously submitted Exhibit B. In view of the foregoing, and that all relevant information concerning source and relevancy of the data is provided in WO 2010/088739, full consideration of the data is respectfully requested.

WRITTEN DESCRIPTION

The rejection of claims 73, 80, 81, 106 to 112, 115, 116 and 121 to 124 under 35 U.S.C. §112, first paragraph as allegedly lacking an adequate written description is respectfully traversed. According to the Patent Office, allegedly the claims contain subject matter which is not adequately described in the specification to reasonably convey to one skilled in the art that Applicants had possession of the invention.

The claims are adequately described under 35 U.S.C. §112, first paragraph. Nevertheless, solely in order to further prosecution of the application and without acquiescing to the propriety of the rejection, claim 110 has been cancelled herein without prejudice and the rejection is moot. The rejection will therefore be addressed with respect to the amended and new claims.

Applicants reiterate that claims 80 and 81 recite the light and heavy chain variable regions of SAM-6, namely SEQ ID NOs:1 and 3, respectively. Consequently, as the antibodies and functional fragments are defined by SEQ ID NOs:1 and 3, claims 80 and 81 are adequately described under 35 U.S.C. §112, first paragraph.

Applicants' representative appreciates the Examiner's suggestion to review TC1600 presentations concerning subject matter related to claiming antibodies. However, Applicants' representative respectfully points out that these are simply guidelines and not controlling authority. As such, Applicants will discuss the facts of the current claims in view of 35 U.S.C. §112, first paragraph and relevant case law.

As a first issue, the Examiner is again requested to review WO 2010/088739, which discloses numerous sequence variants, fragments (scFV, diabodies) and isotype switched forms of SAM-6 that bind to antigen. In this published application, variants and fragments that bind to target were produced based in part on the knowledge in the art concerning antibody structure correlating with function at the time of the invention combined with skillful selection of one or more amino acid substitutions or fragments. Thus, the notion that one of skill in the art would not reasonably have known of variant sequences having a high probability of binding to target, in spite of the knowledge and skill in the art concerning antibody structure and function at the time of the invention, is clearly incorrect in view of the numerous sequence variants, fragments (scFV, diabodies) and isotype switched forms of SAM-6 described in WO 2010/088739 that bind to antigen. Applicants respectfully request that the Examiner give full consideration to the numerous SAM-6 sequence variants, fragments (scFV, diabodies) and isotype switched forms disclosed in WO 2010/088739 that bind to antigen.

Second, Applicants note that *Ariad Pharmaceuticals Inc. v. Eli Lilly & Co.*, 598 F.3d 1336 (Fed. Cir. 2010), is cited in the Action. Applicants reiterate that neither *Ariad* nor any other controlling case law requires an actual reduction to practice or disclosure of a specific number of examples within the scope of the claims to satisfy the written description requirement under 35 U.S.C. §112, first paragraph. *In re Angstadt*, 537 F.2d 498, 502-503 (CCPA 1976), *Utter v. Hiraga*, 845 F.2d 993, 998-99 (Fed. Cir. 1988). In *Ariad* in particular, the court stated that in reference to written description, "[t]he doctrine never created a heightened requirement to provide a nucleotide-by nucleotide recitation of the entire genus of claimed genetic material; it has always expressly permitted the disclosure of structural features common to the members of the genus" Id. at 1352, citing *Regents of the University*

of California v. Eli Lilly and Co., 119 F.3d 1559, 1569 (Fed. Cir. 1997) and *Invitrogen Corp. v. Clontech labs., Inc.* 429 F.3d 1052, 1073 (Fed. Cir. 2005).

The cited *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956 (Fed. Cir. 2002) case is also particularly instructive, since it is also cited in the Action and the court held that the claims of the patent at issue satisfied written description under 35 U.S.C. §112, first paragraph. Again, as in *Ariad*, in *Enzo* the court stated that "[i]t is not correct, however, that all functional descriptions of genetic material fail to meet the written description requirement." *Enzo* at 964.

Thus, in *Ariad* as in *Enzo* there is no requirement that claims directed to antibodies require the sequence of all antibodies, or the antigen, in order to meet the written description requirement. Furthermore, even where the antigen is not fully defined by sequence, the facts here are that 1) the antigen need not be defined by sequence in order to identify binding antibodies and fragments (as corroborated by Exhibit D, excerpted from WO 2010/088739); and more significantly 2) the claimed genus of antibodies and functional fragments is clearly defined and is highly conserved functionally, and is clearly defined and highly conserved structurally owing to substantial sequence identity to reference sequences.

To reiterate, in addition to sharing the functional feature of binding to a common antigen expressed by at least one well-defined cancer cell line, and binding to the antigen to which SAM-6 defined by the amino acid sequences of SEQ ID NOs:1 and 3 bind, the claimed antibodies and functional fragments also are described structurally. In this regard, antibodies that bind to a common epitope typically share sequence homology. Furthermore, the claims specifically require substantial sequence identity to either the predicted CDRs of SEQ ID NO:3, or substantial sequence identity to SEQ ID NO:3 in its entirety. Thus, the claimed antibodies and functional fragments share both common functional (bind to a common epitope) and substantial structural (sequence identity) relationships with SAM-6 that are highly conserved both functionally and structurally. Accordingly, the facts of the claimed antibodies and fragments, in which the claimed sequences are defined functionally and are highly conserved structurally, and the substantial knowledge of structure correlating with function at the time of the invention, are analogous to the facts in *Invitrogen Corp. v. Clontech Laboratories, Inc.*, 429 F.3d 1052 (Fed. Cir. 2005). Consequently, the claims meet the written description standard set forth in *Ariad*, *Enzo* and *Invitrogen*.

Third, owing to the substantial knowledge and skill in the art in terms of antibody structure correlating with function at the time of the invention, and that the claims all require

substantial sequence identity to either the predicted CDRs of SEQ ID NOs:3, or substantial sequence identity to SEQ ID NO:3, the skilled artisan would also have known residues within SEQ ID NOs:1 and 3 more and less amenable to substitution. Consequently, one skilled in the art could have been able to predict with a high degree of confidence many substitutions of SEQ ID NOs:1 and 3 that would not destroy binding activity, as corroborated by the numerous SAM-6 sequence variants, fragments (scFV, diabodies) and isotype switched forms described in WO 2010/088739 that bind to antigen.

Lastly, the facts in the cited *Noelle v. Lederman*, 355 F.3d 1343 (Fed. Cir. 2004) case are clearly distinguishable from the facts of the claimed antibodies and fragments. In *Noelle* the claims at issue were directed to a broad genus of antibodies, including mouse, humanized and human forms of monoclonal antibodies that bound to CD40CR. However, neither human CD40CR antibody nor human CD40CR antigen were described in the patent in suit and the *Noelle* court held that disclosure of a murine CD40CR antigen and murine CD40CR antibody did not provide written description for human CD40CR antibody. Furthermore, in *Noelle* it was determined that one of skill in the art would not have had a reasonable expectation of isolating activated T cells (it would have been "extremely difficult") that produced the required human CD40CR antigen for producing a human CD40CR antibody. In contrast to *Noelle*, the claims are directed to a concise genus of antibodies and fragments having conserved function and a substantial degree of sequence identity to an exemplary reference antibody that binds to the same target antigen expressed by at least one of several well characterized cancer cell lines. Moreover, all of the exemplified antibodies in the as-filed specification are human in origin and the murine –human distinction of the *Noelle* case is simply inapposite to the facts of Applicants' claims. Also in contrast to *Noelle* which disclosed no human CD40CR antibody, the variable region sequences (SEQ ID NOs:1 and 3) of an exemplary antibody are disclosed in Applicant's specification, and given the substantial knowledge of structure/function correlations in the antibody art at the time of the invention, one skilled in the art could produce variant antibodies and fragments that would likely retain binding activity.

In view of the foregoing and the reasons of record, the claims are adequately described under 35 U.S.C. §112, first paragraph. Accordingly, Applicants respectfully request that the rejection be withdrawn.

The new grounds for rejection of claims 111 and 124 under 35 U.S.C. §112, first paragraph as allegedly lacking an adequate written description is respectfully traversed. According to the Patent Office, allegedly the correction of 2 incorrect amino acid residues in SEQ ID NO:3 from Lys-Thr to Arg-Pro is new matter.

As discussed above, the court in *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956 (Fed. Cir. 2002) held "that reference in the specification to a deposit may also satisfy the written description requirement with respect to a claimed material," and that "reference in the specification to deposits of nucleotide sequences describe those sequences sufficiently to the public for purposes of meeting the written description requirement." Id. at 965 and 970. In view of the fact that a deposit of sequences satisfies the written description requirement for those sequences, the referenced deposited biological material (DSM ACC2903) in the specification adequately describes the recited heavy (SEQ ID NO:3) and light chain (SEQ ID NO:1) variable region sequences. Accordingly, the amendment correcting the sequence error of SEQ ID NO:3 from Lys-Thr to Arg-Pro does not add new matter, and the rejection under 35 U.S.C. §112, first paragraph, must be withdrawn.

CONCLUSION

Please charge any fees associated with the submission of this paper to Deposit Account Number 033975. The Commissioner for Patents is also authorized to credit any over payments to the above-referenced Deposit Account.

Respectfully submitted,

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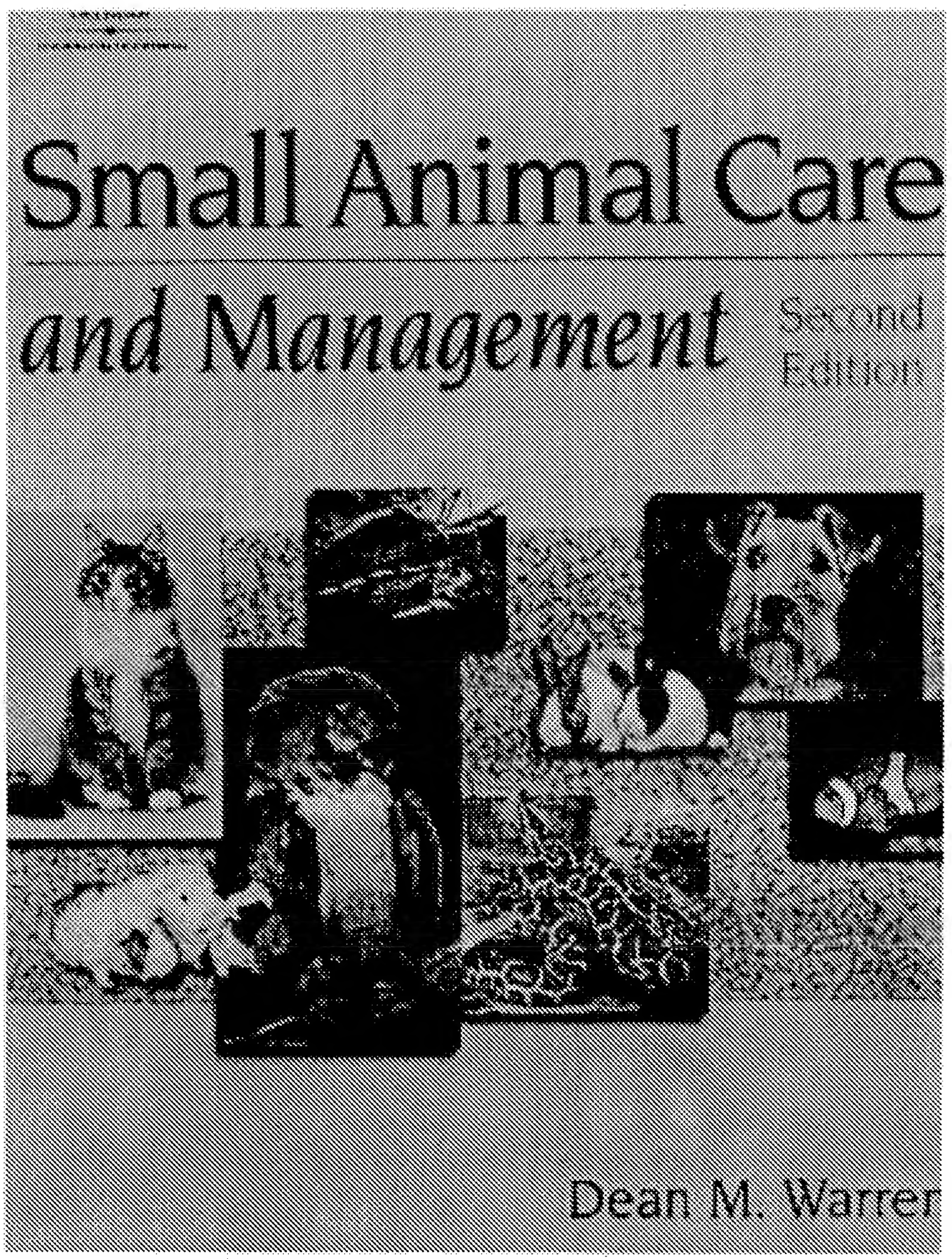
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overheated. The cells of the body are starved for oxygen, and there is a slowdown in normal body functions. There is also a lowering of the body's resistance to disease.

Water is quickly absorbed into the body through the walls of the stomach. It is important that the animal receive a supply of good clean, fresh water. Any disease organisms in the water are also quickly absorbed into the animal's body, which creates a possibility of infection. Water is also a good carrier for drugs or chemicals for disease control.

Water makes up about 55 to 65 percent of an animal's body. A beef steer has about 55 percent of its body composition as water, whereas a mouse has about 65 percent. The composition of an animal's blood is 90 to 95 percent water, muscle is 72 to 78 percent water, and bone is 30 to 40 percent water.

The amount of the essential amino acids needed varies depending on the animal's function and stage of growth.

Carbohydrates

Carbohydrates contain the chemical elements carbon, hydrogen, and oxygen. Animals convert carbohydrates into energy that is needed for the following:

1. supporting bodily functions, such as breathing, digestion, and exercising
2. producing heat to keep the body warm
3. storing fat

Carbohydrates are made up of the group of chemicals called sugars, starches, and crude fiber. These

groups are usually classified as nitrogen-free extract (N.F.E.) and crude fiber on most feed bags. Nitrogen-free extracts are the more easily and completely di-

Vitamins D, D_2, and D_3 are associated with the use of calcium and phosphorus in the body. Animals deficient in vitamin D usually have weak trembling legs.

Human hybridoma produced protein (Patrys GmBH, Germany). The gene sequence for this protein differs from the PAT-SM-6 IgM in the VL domain, based on protein sequence by Mass Spec.

SAM-6 1.1B scFv has a the same VH sequence as 1.1A, but a single amino acid change in the VL domain

SAM-6 2.2B scFv has 2 framework and 1 VH CRD2 changes, and single amino acid change in the VL domain

Binding to LDL was used to measure protein expression of the various scFv sequences by bacteria. The data reveal that the strongest signal is observed with the SAM-6 KTA and SAM-6 2.7, indicating that these two variants were produced in higher amounts. The variant residues in each will be combined to incorporate these amino acid changes in an IgG variant of SAM-6.

Example 11

This example includes a description of binding studies showing that SAM-6 diabodies bind to an antigen in conditioned media from A549 cells, and bind to LDL (low density lipoprotein). This example also includes data indicating that certain SAM-6 variants are produced in greater amounts when expressed in cells.

In the ELISA (Figure 24), the two bars to the right show binding of scFv 1.1A and 1.1B to LDL. In brief, LDL is coated onto the plate, then the plate is blocked. SAM-6 diabodies (with FLAG tag at C-terminus) are added (incubated) then the unbound protein is washed away. Another antibody is added that binds to the FLAG tag that is also coupled to HRP (HorseRraddish Peroxidase). This binding can be detected in a colour reaction that is recorded by the ELISA plate reader. An absorbance reading around 1.0 indicates that the proteins are binding.

SAM-6 binds to the cancer cell line A549, so these cells produce target in an accessible form that the antibody binds to, most likely on the cell surface. A549 cells were grown and by day three the cells have formed a confluent layer on the bottom of the tissue culture flask. Spent culture media (now called conditioned media as it now has the A549 cell growth by-products in it, including fetal calf serum– as well as the secreted target protein, but no A549 cells as they are removed by centrifugation) were collected and coated to ELISA wells. The neighbouring (control) well had growth media that never had any cells growing in it. After blocking, the SAM6-diabodies are added and allowed to bind. Unbound protein is washed away and the secondary antibody added for flag tag detection.

EXHIBIT D

The two conditioned media bars show that A549 cells produce material in the medium to which SAM-6 antibody binds (Figure 24). The A549 cells produced an antigen target that is apparently secreted into the culture media. This data indicates that there is a large amount of target (antigen) protein in the A549 conditioned media.

The binding to LDL data indicate that scFv KTA (SAM-6 old) with two residue change in CDR3 had greater relative binding to LDL than other SAM-6 variants. The data also reveal that a change of the framework region in scFv 2.7A also had greater relative binding affinity for LDL than other SAM-6 variants. A summary of the rank order for binding to LDL for scFv variants was:

KTA (SAM-6 old) better than 1.1A (B, CDR3)
2.7A better than 1.1A (F)
1.1A = Percivia expressed (PAT-SAM-6)
1.1B less binding than 1.1A (F)
2.2B less than 1.1A (F,B)
1.5A less than 1.1A (B)
1.4A less than 1.1A (F,B, CDR3)
1.2A less than 1.1A (B)
2.2A less than 1.1A (F,B)
(F)= Framework change; and (B)= Binding region change

Thus, combining the KTA (SAM-6 old) and the 2.7A framework should result in improved binding affinity for LDL.

Example 12

This example includes a description of studies showing that SAM-6 diabodies bind to an antigen in conditioned media from A549 cells, and bind to a cell free translated non-glycosylated grp78 protein (using wheat germ).

ELISA assays were performed. Antigen was coated at 0.5 µg/well in a volume of 50µl/well. Primary antibody was used at 12µg/ml (0.6µg/well). Buffer was pH6.5 and the dilution buffer used was a high salt pH6.5. Grp78 protein was diluted with 1 x PBS pH7.4

ELISA demonstrated binding to a component of A549 cancer cell culture media (after cells were grown for three days). A549 conditioned media (cells removed) was coated onto the entire plate. The SAM-6 diabodies were added (1.1 and KTA and optimised). Other control antibodies were

included, an LM1 diabody, CM1 diabody, a control VH dimer-which is actually a monomer, a BARB3-diabody, BARB4 diabody, a recombinant PAT-SAM6 450-IgM (produced by PerC6 cells, Percivia), recombinant LM1 41B1-IgM , and SAM6 C8/9 hybridoma IgM. The negative controls are the conditioned media that the A549 cells have been growing in, without the primary antibody and with the secondary antibody. The data show that all of the SAM-6 diabodies bind to an antigen present in the A549 culture supernatent, it is also detected by the recombinant SAM-6 IgM clone 450 (produced by PerC6 cells, Percivia). SAM-6 hybridoma C8/9 gives a very poor signal, but this may be due to protein degradation or hybridoma cell death. This ELISA also shows that the other antibodies tested do not bind to any secreted product in the A549 conditioned media. Only SAM-6 detectably binds to the A549 conditioned media. This ELISA shows that SAM-6 binds to a target in A549 cell conditioned media.

Conditioned media from a second cell line HDFa previously shown not to exhibit cell surface binding to SAM-6 antibodies was studied for binding to SAM-6 antibodies. No binding was detected indicating that this cell line is a good negative control.

Another ELISA was performed on a plate coated with Grp78 protein (from Abnova- cell free protein translation-using wheat germ-non-glycosylated). Binding of recombinant PAT-SAM-6 IgM antibody (clones 450 and 528 produced by PerC6 cells, Percivia) and recombinant SAM-6 1.1A diabody to pure non-glycosylated Grp78 protein was detected. All binding was to pure (non-glycosylated) target Grp78 protein. This data indicates that SAM-6 antibodies and variants bind to grp78 without a carbohydrate moiety. On the second half of the plate binding to conditioned media from A549 cells was detected, whereas negative control LM1 antibodies did not bind to the conditioned media. There was variation in the strength of the signal detected but the target protein may not be uniformly dispersed throughout the sample.

Example 13

This example includes a description of studies showing various forms of SAM-6, including SAM-6 scFv, SAM-6 variants and SAM-6 heavy chain variable region (V_H) alone, without light chain variable region (V_L) bind to an apoB100, protein, LDL, VLDL and deglycosylated LDL.

Antigen specificity: Fresh batches of recombinant protein were made and tested against a panel of proteins to determine specificity for LDL. The ELISAs were repeated several times. Positions of the antigens on the plates were randomized to rule out position effects.

For SAM-6 KTA scFv, antigens were coated at 0.5ug/well, volume 50ul/well. Buffer 1 x PBS pH 6.5 Primary antibody SAM-6 KTA scFv affinity purified (anti-HIS-denatured) soluble C dialysed and added neat (50ul/well) (2BTA46). Note that in these protein samples the soluble B fraction has been removed and only the remaining soluble C fraction is tested. The 3rd time reading are higher as they contain the combined protein level (1CHO4.8) from the urea solubilised extraction. The positive control antibody anti-Lewis Y, was anti-FLAG purified and added neat (50ul/well) (2BTA49). The well contains the antigen, Lewis Y tetrasaccharide bound to HSA(Human Serum Albumin). The positive control (anti-Lewis Y) gave an absorbance reading at A655nm of 0.98 on one ELISA and 0.90 on the other ELISA when binding to its carbohydrate antigen lewis Y.

The strongest binding of SAM-6 KTA scFv is to Apolipoprotein B100. Binding to VLDL, LDL and deglycosylated LDL was also detected.

For SAM-6 1.1A scFv urea solubilized, antigens were coated at 0.5ug/well, volume 50ul/well. Buffer 1 x PBS pH 6.5 Primary antibody SAM-6 1.1A scFv affinity purified (anti-HIS-denatured) soluble C dialysed and added neat (50ul/well) (2BTA46). The 3rd time readings contain the combined protein level (1CHO4.7) from the urea solubilised extraction. The positive control antibody anti-Lewis Y, was anti-FLAG purified and added neat (50ul/well) (2BTA49) gave an absorbance reading at A655nm of 1.2 and 1.0.

Strong binding of SAM-6 1.1A to Apolipoprotein B100, VLDL, LDL and de-glycosylated LDL was detected.

For SAM-6 (Percivia), strong binding to Apolipoprotein B100, VLDL, LDL and de-glycosylated LDL was detected.

For SAM-6 HAB produced by human hybridoma (Patrys GmBH, Germany), strong binding to VLDL, LDL and de-glycosylated LDL, was detected but less binding to apolipoprotein B100. The SAM-6 HAB gave variable results in this assay.

In the foregoing studies several different SAM-6 proteins produced in a variety of different formats were compared for their ability to bind to various target antigens, such as LDL (Low Density lipoprotein), VLDL, deglycosylated LDL and apoB100 protein. SAM-6 KTA scFv, 1.1 scFv, PAT-SAM-6 (Percivia) and SAM-6 HAB exhibited various degrees of binding affinity for LDL, VLDL, deglycosylated LDL and apoB100 protein, but not HDL (high density lipoprotein). In this way sequence changes can be linked to function.

Further binding studies to ApoB100 were performed by ELISA analysis. In brief, 250ul of Apolipoprotein B100 (10ug/ml) isolated from low density LDL (purchased from Calbiochem) was coated onto ELISA plates. Plates were blocked, incubated with primary single-chain antibodies (SAM-6.2.7 and SAM-6.opti) and SAM-6 heavy chain variable region (V_H) alone), and then incubated with anti-FLAG-HRP secondary antibody in a total volume of 250ul, and compared to three negative controls (Negative control 1: No coating (blocked), No primary, then anti-FLAG-HRP secondary; Negative control 2: No coating (blocked), then primary, then anti-FLAG-HRP secondary; and Negative control 3: Coated with 10ug/ml ApoB100 (blocked), No primary, then anti-FLAG-HRP secondary).

The results indicated that SAM-6.2.7, SAM-6.opti and SAM-6 heavy chain variable region (V_H) alone) bind to ApoB100 protein.

Example 14

This example includes a description of studies showing that SAM-6 variants can also bind to cancer cell lines A549, BxPC3 and CRL1424.

The following variants were studied: SAM-6 1.1A scFv, SAM-6 KTA scFv, SAM-6 VHVL opt scFv, SAM-6 HAB, and PAT-SAM-6 (Percivia). SAM-6 VHVL opt scFv has an optimized framework with 4 amino acid changes in the VH domain including 25% changes at the nucleotide level. There is one additional change in CDR-H1. The VL domain of SAM-6 VHVL opt scFv is a class switch from lambda to kappa light chain with 40 amino acids changed including 38% changes at the nucleotide level. The free Cys residue was removed from the VL CDR1.

FACS analysis revealed that all of the scFv constructs bind to the three cancer cell lines tested (A549, BxPC3 and CRL1424), but not to the negative cell line HDFa.

Additional studies were performed using confocal microscopy analysis for binding to A549, BxPC3, CRL1424, HT-29, HeLa, and MCF-7 cancer cell lines. The SAM-6 antibodies studied included SAM-6 1.1A scFv, SAM-6 KTA scFv, SAM-6 VHVL opt scFv, SAM-6 HAB, and PAT-SAM-6 (Percivia).

In brief, cells were fixed, the primary antibody was added, then detected with a secondary antibody with a FITC label. The cell nucleus was stained with a DAPI stain that appears blue, and measured in the 600-650 wavelength range. This DAPI image was captured and recorded. If the level of DAPI nuclear stain was kept at a constant level, different studies can be "normalized." The cells were incubated with the primary (test) antibody, and the appropriate labelled secondary

antibody added. In these experiments we used an FITC label that was measured at the maximum intensity observed in the 500-550 wavelength range. The FITC image was recorded. The images were overlays of the DAPI image and the FITC images.

In another set of studies the ability of the proteins to show two active binding sites was studied. In order to see a positive result, the protein would need to bind to the cell surface target antigen on the cancer cell line, with one binding arm, and then the second arm would be required to bind to the human LDL labelled with Alexa 488. Only when these two events occur would a binding event be detected. The Alexa 488 image does not fade and is more stable and we found that it generated a more intense staining image.

The results revealed binding was detected for A549 (Lung), BxPC-3 (pancreatic), HT-29 (colon), HeLa (cervix), MCF-7 (breast) and CRL1424 (melanoma) cells for all five SAM-6 antibodies. Differences were observed in the binding of the SAM-6. In some cell lines it appears that the SM-6 proteins have entered the cell nucleus, when the overlays are done the cell nucleus appears a lighter but brighter blue. None of the five SAM-6 antibodies detectably bind to stomach cancer cell line 23132/93.

Example 15

This example includes a description of additional studies with IgG1 SAM-6 variants, which can also bind to cancer cell line A549.

SAM-6 scFv's were converted into IgG1 using the lambda light chain and the IgG protein expressed in mammalian cells HEK293F. To date, the three different SAM-6 proteins produced in IgG1 format are SAM-6 1.1imp IgG, which contains the 1.1A VH domain with one amino acid change to improve expression, now called 1.1imp. It also contains the CH1-CH2-CH3 of IgG1. The light chain is the same lambda light chain used in the recombinaint IgM construct. SAM-6 KT imp IgG, which contains the KTA VH domain with one amino acid change to improve expression and the CH1-CH2-CH3 of IgG1. The light chain is the same lambda light chain used in the recombinaint IgM construct. SAM-6 opt IgG, which contains the S6 optimised VH domain with four amino acid changes as well as codon optimisation to improve expression and the CH1-CH2-CH3 of IgG1. The light chain is the same lambda light chain used in the recombinant IgM construct. KTA means that the KT VH is paired with the 1BTA1.6 VL domain in the scFv construct.

Culture supernatant was isolated after transient transfection of HEK293F cells. At day 5, cell supernatant with the appropriate IgG1. Binding of SAM-6 1.1imp IgG1, SAM-6 KT IgG1 and SAM-6 opt IgG1 to A549 cells was detected.

Additional FACS studies were performed with HeLa cells with SAM-6 1.1imp IgG1 and SAM-6 Kopti IgG1. Binding of SAM-6 1.1imp IgG1 and SAM-6 Kopti IgG1 to HeLa cells was detected.

Example 16

This example includes a description of studies showing that alterations of amino acid residues in SAM-6 scFvs can increase protein solubility.

The limited solubility of scFv's antibodies, as seen with SAM-6 1.1A, whose Fv sequence is unchanged from parent PAT-SAM-6 IgM, has limited storage and reduced efficiency in trials of the antibody over time. However, as shown in the Table below, with SAM-6 optimized (scFv dimmer) and SAM-6 opt. scFv monomer, codon usage optimization combined with targeted residue changes in the VH domain as well as a new VL domain framework all contribute to improved protein solubility.

Solubility of scFv's in Biological Buffers

scFv antibody	Concentration from Profinia™ (containing 6M Urea)	Concentraion post dialysis (BCA)	Percent recovery after dialysis
SAM-6 1.1A	1.100 mg/ml	0.174 mg/ml	16%
Optimized dimer	1.200 mg/ml	0.861 mg/ml	72%
PAT-SAM-6 opt monomer	1.300 mg/ml	1.223 mg/ml	94%

The data in the table indicate that SAM6 optimized (diabody) vs SM-6 opt (optimized) monomer have a higher yield than SAM-6 1.1A.

Example 17

This example includes a description of studies showing ELISA binding studies of SAM6-IgM to lipoprotein (LDL) and an apoptosis assay.